

A Novel, Comparative Study of Chemical and Green Synthesis of Silver Nanoparticles

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Abstract

Silver Nano particles (AgNps) have attracted increasing interest due to their unique physical, chemical and biological properties compared to their macro scale counterparts. Green synthesis of silver Nano particles (AgNps) are prepared by aqueous solution of silver nitrate and extract of kaffir lime fruit (*citrus hystrix*), which contain relatively high amount of ascorbic acid. The chemical approach is done by the aqueous solution of silver nitrate and tri- sodium citrate. The synthesized silver nano particles were characterized by Fourier transform infrared spectroscopy (FTIR) analysis. Anti-bacterial activity of the synthesized silver Nano particles were tested against both gram positive and gram negative bacterial strains.

Keywords: Kaffir lime; Silver Nano particles; Tri- sodium citrate.

1. INTRODUCTION

In recent days nanotechnology has induced great scientific advancement in the field of research and technology. Nanotechnology is the study and application of small object which can be used across all fields such as chemistry, biology, physics, material science and engineering. As the name indicates Nano means a billionth or 10^{-9} unit. Its size range usually from 1-100 nm due to small size it occupies a position in various fields of nano science and nanotechnology (Bharathi *et al.* 2014; Anil Ramdas Shet *et al.* 2015). Nano size particles are quite unique in nature because nano size increase surface to volume ratio and also its physical, chemical and biological properties are different from bulk material. Thus in recent years much research is going on metallic nanoparticle and its properties like catalyst, sensing to optics, antibacterial activity, data storage capacity ((Bharathi *et al.* 2014).

A quest for an environmentally sustainable synthesis process has led to a few bio-mimetic approaches. Sometimes the synthesis of nanoparticles using plants or parts of plants can prove advantageous over other biological processes by eliminating the elaborate processes of culturing of micro-organisms (Anil Ramdas Shet *et al.* 2015). Among metal nanoparticles, silver nanoparticles (AgNPs) have been known to have inhibitory and antimicrobial activity. Considering the antioxidant properties of fruit juices, we have made an attempt to use fruit juice as conglomerate of metabolites as reducing and stabilizing agent for the

biosynthesis of metal nanoparticles (Basavaraj S. Hungund *et al.* 2015).

Silver nano particles (AgNPs) have attracted increasing interest due to their unique physical, chemical and biological properties compared to their macro-scaled counterparts (Krishnamoorthy and Jayalakshmi, 2015). AgNPs have distinctive physico-chemical properties, including a high electrical and thermal conductivity, surface-enhanced Raman scattering, chemical stability, catalytic activity and nonlinear optical behaviour (Basavaraj S. Hungund *et al.* 2015). These properties make them of potential value in inks, microelectronics, and medical imaging. There are different methods of synthesis of metallic nanoparticles, which are chemical, physical, biological methods, etc.,. In the present study AgNPs were synthesised by chemical reduction and green synthesis methods (Raid Salih Jawaad *et al.* 2014). The synthesised samples were further characterised using FTIR analysis and *invitro* antimicrobial activities were performed against both Gram positive and Gram negative micro-organisms such as *Bacillus cereus*(MTCC 430), *Staphylococcus aureus* (MTCC 3160), and *E.coli* (MTCC 1698),*Klebsiella pneumonia* (MTCC10309).

2. MATERIALS & METHODS

2.1 Materials

All analytical reagents and media components were purchased from Merck and Sigma Chemicals.

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2.1.1 Preparation of fruit juice extract

The fruit selected for the synthesis of silver nanoparticles is *Kaffir lime* (*citrus hystrix*). The fresh fruit was collected from the erode region. Fresh fruit extract were prepared by slicing the fruit into two pieces. From the slices the pulps were extracted and centrifuged at 5000 rpm for 10 mins. Then the fresh extract can be collected and stored in refrigerator for further uses.

2.1.2 Preparation of silver nitrate solution

Molecular weight of AgNO_3 is 167.89 g. In order to prepare 0.01M silver nitrate solution 0.08 g is added to 100 ml distilled water.

2.2 Synthesis of silver nanoparticles by tri-sodium citrate

Silver Nanoparticles were prepared by chemical reduction method. 50 ml of 0.01 M Silver nitrate was heated. To this solution 10 ml of 0.01M tri-sodium citrate was added drop by drop. During this process solution was mixed vigorously and heated until colour change was obtained (yellowish brown), indicates the presence of Silver Nanoparticles.

2.2.1 Synthesis of silver nanoparticles using fruit extract

Silver nanoparticles were synthesized by *Kaffir lime* fruit extract. 12.5 ml of fruit extract and 50 ml of 0.01M AgNO_3 solution was mixed and then constantly stirred. After 30mins there will be a change in solution colour. The colour changes indicate the formation of silver Nano particles. As the fruit extract was mixed in the aqueous solution of silver ion complex, it started to change the colour from white to yellowish brown due to reduction of silver ion which may be the indication of formation of silver nanoparticles.

3. CHARACTERIZATION TECHNIQUES

3.1 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis of the Synthesised silver nanoparticles using chemical reduction and green synthesis methods were observed by **Perkin Elmer Spectrum One FTIR** with spectral range of 4000-400 cm^{-1} using KBr pellet.

3.2 Invitro antimicrobial activity

Antimicrobial activity of the synthesised silver nanoparticles using chemical reduction and green synthesis methods were assessed using the standard well diffusion method with help of Blood Agar Base medium. Liquid nutrient agar media and the Petri plates were sterilized by autoclaving at 121 °C for about 30 min at 15 lbs pressure. Under aseptic conditions in the laminar airflow chamber, about 20 ml of the agar

medium was dispensed into each Petri plate to yield a uniform depth of 4mm. After solidification of the media, 24 hrs culture of Gram positive microorganisms such as *Bacillus cereus* (MTCC 430), *Staphylococcus aureus* (MTCC 3160), Gram negative microorganisms such as *E.coli* (MTCC 1698) and *Klebsiella pneumonia* (MTCC10309) obtained from IMTECH, Chandigarh were swabbed on the surface of the agar plates. Well was prepared by using cork borer followed with loading of 100 μl of each sample to the distinct well with sterile distilled water as negative control and Tetracycline (30 mcg/disc) as positive control. The sample loaded plates were then incubated at 37°C for 24 hours to observe the zone of inhibition.

4. RESULTS & DISCUSSION

4.1 FTIR Analysis

The FTIR spectra of the synthesised samples are given in fig.1 & 2. (here after named as Ag1 and Ag2). In fig.1 & 2 the absorption peak at 3866 cm^{-1} and 3415 cm^{-1} are assigned to -OH stretching in alcohols and phenolic compounds (Basavaraj S. Hungund *et al.* 2015). The peak absorbed at 3743 cm^{-1} assigned to the C-H stretching vibrations of methyl, methylene and methyl groups. For sample Ag1 peak observed at 1640 cm^{-1} and for sample Ag2 at 1635 cm^{-1} that could be attribute to the stretching vibrations of C=C. peak observed at 1021 cm^{-1} for both Ag1 and Ag2 ascribed to multiplet C=O group. The spectra exhibits the presence of silver nanoparticles.

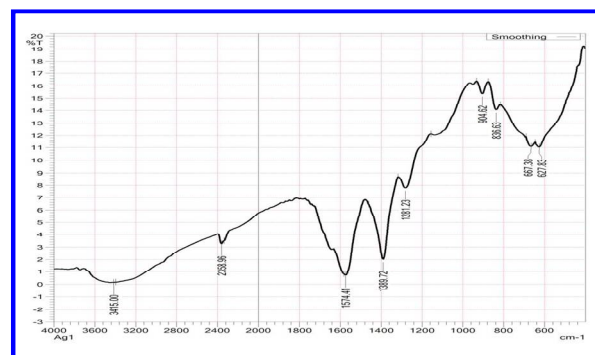


Fig. 1: FTIR spectra of silver nanoparticles AgNPs synthesized using tri sodium citrate (Ag1)

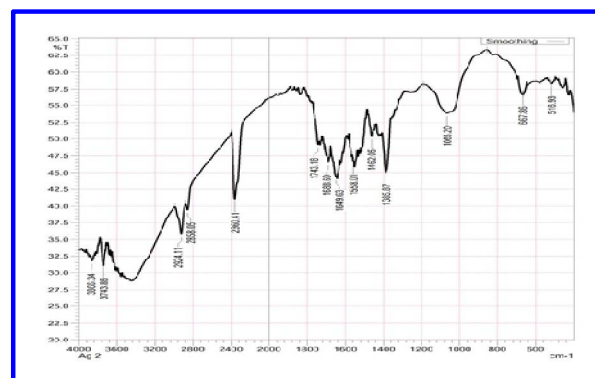


Fig. 2: FTIR spectra of silver nanoparticles AgNPs synthesized using kaffir lime extract (Ag2)

4.2 Anti bacterial activity studies

The inhibition zone values are determined for the prepared samples against multidrug resistant human pathogens such as Gram positive micro-organisms such as *Bacillus cereus* (MTCC 430), *Staphylococcus aureus* (MTCC 3160), Gram negative micro-organisms such as *E.coli* (MTCC 1698) and *Klebsiella pneumoniae* (MTCC10309) . The results and images of the inhibition zones are presented as average values mm as shown in Table 1 and in fig 3 & 4 and the diameter of the zones are measured in mm. Ag1 has strongest inhibition against *Escherichia coli* and *Klebsiella pneumoniae* than Ag2. Ag2 has strongest inhibition against *Staphylococcus aureus* than Ag1. Both the sample have strongest inhibition against *Bacillus cereus*.

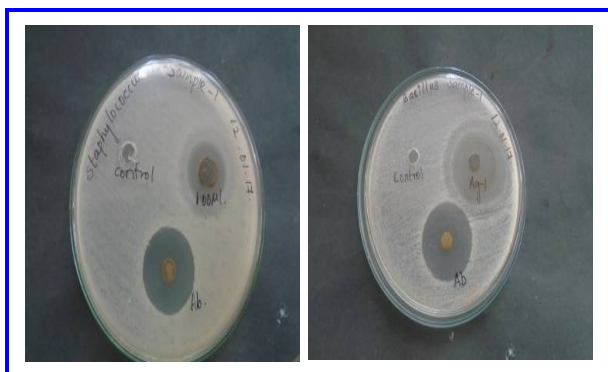


Fig. 3: Antimicrobial study against gram positive bacterial for (Ag1)



Fig. 4: Antimicrobial study against gram negative bacterial (Ag1)

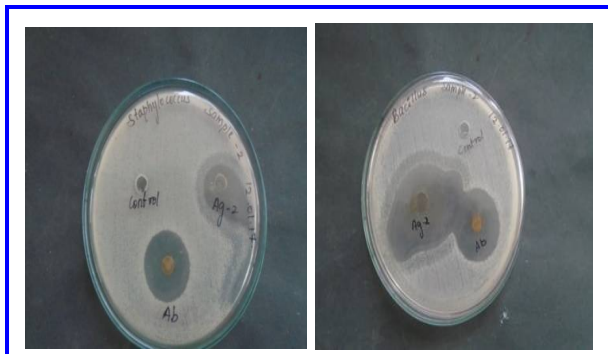


Fig. 5: Antimicrobial study against gram positive bacterial for (Ag2)

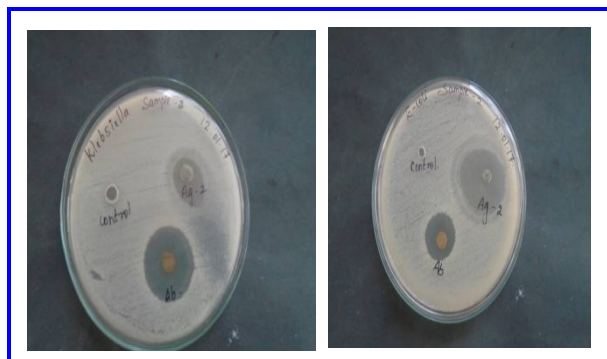


Fig. 6: Antimicrobial study against gram negative bacterial (Ag2)

Table 1. Zone of inhibition of silver nanoparticles (AgNps) synthesized by chemical reduction and green synthesis method

S. No.	Microorganisms	Zone of Inhibition in Diameter (mm)			Std. Antibiotic (Tetracycline) 30mcg/disc
		Control (100 µl)	Ag1 100 µl	Ag2 100 µl	
1	<i>Bacillus cereus</i>	Nil	31	31	27.5
2	<i>Staphylococcus aureus</i>	Nil	19	32	26
3	<i>Escherichia coli</i>	Nil	38	33	20
4	<i>Klebsiella pneumoniae</i>	Nil	28	16	23

5. CONCLUSION

In the present study, silver nano particles were synthesised by chemical reduction and green synthesis method. The functional group analysis depicts the presence of silver in both synthesised methods. Invitro antimicrobial Study reveals that the synthesised samples have antibacterial activity against both gram positive and gram negative bacterial strains, whereas, Ag1 has strongest inhibition against *Escherichia coli* and *Klebsiella pneumoniae* than Ag2. Ag2 has strongest inhibition against *Staphylococcus aureus* than Ag1. Both the sample have strongest inhibition against *Bacillus cereus*.

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